

Claims

1. A method to isolate at least one target molecule of a compound comprising a functional group that can be specifically altered, said method comprises the following steps:
 - (a) adding said compound to a complex mixture of molecules wherein said compound stably interacts with at least one molecule forming a compound-target complex,
 - (b) separating the resulting complex mixture of molecules and compound-target complexes into fractions via chromatography,
 - (c) chemically, or enzymatically, or chemically and enzymatically altering said compound present on at least one compound-target complex in each fraction, and
 - (d) isolating at least one target molecule that interacts with said compound via chromatography, wherein the chromatography of steps (b) and (d) is performed with the same type of chromatography.
2. The method of claim 1, wherein the chromatographic conditions of steps (b) and (d) are the same or substantially similar.
3. A method according to claims 1 or 2 wherein said complex mixture of molecules is a complex mixture of proteins.
4. A method according to claim 3 further comprising the cleavage of said complex protein mixture into a protein peptide mixture before performing step (b).
5. A method according to claims 1 or 2 wherein said complex mixture of molecules is a protein peptide mixture.
6. The method of claims 1 to 5, further comprising the step of identifying the targets.
7. The method of claim 6, wherein said target molecules are proteins or peptides and wherein said identifying step is performed by a method selected from the group consisting of: a tandem mass spectrometric method, Post-Source Decay analysis, measurement of the mass of the peptides, in combination with database searching.
8. The method of claim 7, wherein said identifying step based on the mass measuring of the target peptides is further based on one or more of the following: (a) the determination of the number of free amino groups in the target peptides; (c) the knowledge about the cleavage specificity of the protease used to generate the protein peptide mixture; and (d) the grand average of the hydropathicity of the target peptides.
9. A method to determine the relative amount of the level and/or activity of at least one target protein in more than one sample comprising proteins, comprising the steps of: (a) the addition of a compound comprising a first isotope to a first sample comprising peptides wherein said compound stably interacts with at least one peptide forming a compound-peptide complex; (b) the addition of a compound comprising a second isotope to a second sample comprising peptides wherein said compound stably interacts with at least one peptide forming a compound-peptide complex; (c) combining the protein peptide mixture of

the first sample with the protein peptide mixture of the second sample; (d) separating the combined protein peptide mixtures into fractions of peptides via chromatography; (e) chemically, or enzymatically, or chemically and enzymatically, altering said compound present on at least one compound-peptide complex in each fraction; (f) isolating the altered compound-peptide complexes out of each fraction via chromatography, wherein the chromatography is performed with the same type of chromatography as in step (d); (g) performing mass spectrometric analysis of the isolated altered compound-peptide complexes; (h) calculating the relative amounts of the altered compound-peptide complexes in each sample by comparing the peak heights of the identical but differentially, isotopically labelled altered compound-peptide complexes, and (i) determining the identity of said peptides in the altered compound-peptide complexes and their corresponding proteins.

10. The method according to claim 9 wherein the chromatographic conditions of steps (d) and (f) are the same or substantially similar.

11. The method of claim 9 or 10 wherein the determining of the identity of the altered compound-peptide complexes is performed by a method selected from the group consisting of: the tandem mass spectrometric method, Post-Source Decay analysis, measurement of the mass of the peptides, in combination with database searching.

12. The method of claim 11, wherein the determining of the identity of the altered compound-peptide complexes is further based on one or more of the following: (a) the determination of the number of free amino groups in the peptides; (c) the knowledge about the cleavage specificity of the protease used to generate the protein peptide mixture; and (d) the grand average of the hydropathicity of the peptides.